

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Marton et al.

Application No.: To be assigned; Division of
Serial No. 09/222,582, filed
December 28, 1998

Group Art Unit: To be assigned

Filed: On even date herewith

Examiner: To be assigned

For: METHODS FOR DETERMINING
THERAPEUTIC INDEX FROM GENE
EXPRESSION PROFILES

Attorney Docket No.: 9301-136

PRELIMINARY AMENDMENT UNDER 37 C.F.R. §1.121

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Pursuant to 37 C.F.R. § 1.121, please enter the following amendments and consider the following remarks in connection with the above-identified application.

IN THE SPECIFICATION:

Please amend the specification as follows:

On page 3, line 11, after the word "understanding" insert --of--.

On page 11, line 18, after "filed on May 8, 1998" insert --, now U.S. Patent No. 5,965,352,--.

On page 12, line 31, delete the word "particular" and insert in its place --particularly--.

On page 24, lines 11-12, delete "in Section 5.2."

On page 25, line 3, delete the word "possible."

On page 29, line 31, delete the word "date" and insert in its place --data--.

On page 30, line 5, delete the word "perturbation" and insert in its place
--permutation--.

On page 30, line 8, delete the word "perturbations" and insert in its place
--permutations--.

On page 35, line 22, after "filed on May 8, 1998," insert --now U.S. Patent No.
5,965,352,--.

On page 36, line 2, delete the word "tl" and insert in its place
--t_l--.

On page 36, line 7, delete the word "both."

On page 37, line 32, after "filed on May 8, 1998," insert --now U.S. Patent No.
5,965,352,--.

On page 39, line 13, delete the word "the" before the word "those."

On page 39, line 17, insert the word --a-- between the word "to" and the word
"patient."

On page 39, line 28, after the word "illustrates" delete the word "the."

On page 40, line 2, replace the word "DICOVER" with the word "DISCOVER."

On page 40, line 24, replace the word "that" with the word "than."

On page 40, line 25, delete the word "gene" and insert in its place
--genes--.

On page 41, line 10, delete the word "week" and insert in its place
--weak--.

On page 45, line 9, delete the term "8C" and insert in its place
--8B--.

IN THE CLAIMS:

Please amend the claims as follows:

Amend claims 1, 12, 23, 24, 34 and 36 as follows:

1. (Amended) A method for evaluating specificity of a drug comprising comparing activity of said drug against its target pathway (D_{target}) in a biological sample and activity of said drug against at least one off-target pathway ($D_{off-target}$) in said biological sample, wherein said D_{target} and $D_{off-target}$ are based on measurements of a plurality of cellular constituents.

12. (Amended) A method for evaluating specificity of a drug comprising:

a) measuring activity of said drug against its target pathway to obtain a target activity

(D_{target});

b) measuring activity of said drug against at least one pathway other than said target pathway to obtain at least one off-target activity ($D_{off-target}$); and

c) determining said specificity by comparing said target activity and said off-target activity;

wherein said D_{target} and $D_{off-target}$ are based on measurements of a plurality of cellular constituents.

23. (Amended) A method of determining therapeutic index of a drug in a biological sample comprising:

determining said therapeutic index according to the formula: $[SI] TI = C_{target} / C_{off-target}$,

wherein C_{target} is a minimum effective concentration needed to induce a threshold response in a target pathway and $C_{off-target}$ is the minimum toxic concentration needed to induce a threshold response in at least one off-target pathway.

24. (Amended) The method of claim 23 wherein said C_{target} and $C_{off-target}$ are measured according to a method comprising:

a) applying a plurality of levels of said drug to said biological sample and measuring a plurality of cellular constituents at each level of said drug in said biological sample to obtain a first profile of graded drug response;

b) applying said plurality of levels of said drug to a test sample, wherein said test sample is the same as said biological sample except that said target pathway is not functional, and measuring a plurality of cellular [constituents] constituents in said test sample at each level of said drug, to obtain a second profile of graded drug response; and

c) determining said C_{target} and $C_{off-target}$ by comparing said first and second profiles.

34. (Amended) A method of determining a therapeutic index of a drug in a biological sample comprising:

a) applying a plurality of levels of said drug to said biological sample;

b) determining a minimum effective concentration (C_{target}) needed to induce a threshold response in a target pathway, wherein said drug exerts its pharmacological activity through said target pathway;

c) determining a minimum toxic concentration ($C_{off-target}$) needed to induce a threshold response in at least one off-target pathway; and

d) determining said therapeutic index according to the formula: $[SI] TI = C_{target} / C_{off-target}$.

target

36. (Amended) The method of claim 35 [herein] wherein said plurality of cellular constituents are transcripts of a plurality of genes.

Add new claims as follows:

64. (New) A method for evaluating specificity of a drug, said method comprising:

(a) decomposing a drug response profile into one or a combination of pathway response profiles, wherein said drug response profile comprises measurements of a plurality of cellular constituents in a biological sample in response to said drug over a plurality of levels of drug exposure, and each said pathway response profile comprises measurements of a plurality of cellular constituents at a plurality of levels of perturbation to a biological pathway; and

(b) comparing, among said one or a combination of pathway response profiles, the pathway response profiles for the one or more biological pathways associated with therapeutic effects of the drug with the pathway response profiles for the one or more biological pathways that are associated with one or more non-therapeutic effects of the drug, thereby comparing activity of said drug on its target pathway (D_{target}) and at least one off-target pathway ($D_{off-target}$) and evaluating specificity of said drug.

65. (New) The method of claim 64, further comprising transforming said levels of drug exposure into said levels of perturbation by a horizontal scaling transformation.

66. (New) The method of claim 65, wherein said horizontal scaling transformation is a linear transformation.

67. (New) The method of claim 65, wherein said decomposing comprises determining said scaling transformation such that said drug response profile is represented by said one or a combination of pathway response profiles.

68. (New) The method of claim 67, wherein said determining is by a method comprising least squares minimizing the residue between said drug response profile and said one or a combination of pathway response profiles.

69. (New) The method of claim 64, wherein values of said measurements of a plurality of cellular constituents have been converted into cellular constituent set values.

70. (New) A method for evaluating specificity of a drug, said method comprising decomposing a drug response profile into one or a combination of pathway response profiles, wherein said drug response profile comprises measurements of a plurality of cellular constituents in a biological sample in response to said drug over a plurality of levels of drug dosage, and each said pathway response profile comprises measurements of a plurality of cellular constituents at a plurality of levels of perturbation to a biological pathway, thereby evaluating specificity of said drug.

REMARKS

The subject application is a divisional application of Application Serial No. 09/222,582, filed on December 28, 1998.

The specification has been amended to correct typographical errors discovered by Attorneys for Applicants during review of the application. The specification has been amended to replace the word "perturbation" by the word "permutation" at page 30, lines 5 and 8, respectively. Support for this amendment is found in the specification at page 29, lines 24-25. The specification has also been amended to correct a typographical error in the identification of Fig. 8B. Support for this amendment is found in the specification at page 39, lines 30-31. The specification has also been amended to update the status of U.S. Patent Application Serial No. 09/074,983, now U.S. Patent No. 5,965,352. A marked version of the paragraphs in the specification which have been amended, with the amendments indicated by

bracketing for deleting and underlining for additions, is attached hereto as Exhibit A. A clean version of the paragraphs in the specification, as amended, is attached hereto as Exhibit B.

Prior to this Preliminary Amendment, claims 1-44 were pending in the application. Claims 1, 12, 23, 24, 33 and 35 have been amended and new claims 64-70 have been added to more particularly point out the invention. Upon entry of the instant Preliminary Amendment, claims 1-44 and 64-70 will be pending. A marked version of the amended claims indicating the changes to the claims is attached hereto as Exhibit C. A clean version of the pending claims, as amended, is attached hereto as Exhibit D.

Claims 1 and 12 have been amended to include the recitation that D_{target} and $D_{off-target}$ are based on measurements of a plurality of cellular constituents. Support for the amendment is found in the specification at, e.g., page 23, lines 31-35; page 35, line 15, through page 36, line 3; and page 38, lines 11-13.

Claims 23 and 33 have been amended to replace the term "SI" with the term "TI." Support for the amendment is in the specification, e.g., at page 38, line 30 through page 39, line 3.

Claims 24 and 36 have been amended to correct typographical errors.

New claims 64-70 have been added to more particularly point out the invention. Support for claim 64 is found in the specification at page 4, lines 17-18; page 23, lines 18-23 and lines 31-35; page 26, lines 1-8; page 35, line 15, through page 36, line 3; and page 38, lines 11-13. Support for claims 65-66 is found in the specification at page 24, line 11 through page 25, line 19. Support for claims 67-68 is found in the specification at page 26, line 14 through page 27, line 21. Support for claim 69 is found in the specification at page 13, lines 5-16. Support for claim 70 is found in the specification at e.g., page 11, lines 9-10 and page 26, lines 1-8.

No new matter has been added. Entry of the foregoing amendments and the following remarks is respectfully requested.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks into the file of the above-identified application.

Respectfully submitted,

Date April 2, 2001

Adriane M. Antler 32,605

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Enclosures

EXHIBIT A: MARKED VERSION OF PARAGRAPHS IN THE SPECIFICATION
U.S. APPLICATION SERIAL NO. To be assigned
(ATTORNEY DOCKET NO. 9301-136)

(as amended April 2, 2001)

Paragraph at page 3, lines 7-14:

In a clinical setting, a physician must select, among several drugs, the most effective and safe drug for the patient. In making this decision, the physician needs to know how an particular patient may respond to a drug. One approach to individualized therapy decision making is through pharmacogenetics which relates individual variation in drug response to genetic variations. Pharmacogenetics promises a better understanding of the relationship between genetic variation and drug responses. However, so far, it has only provided limited information related to about 50 - 100 known drug metabolizing genes. In addition, pharmacogenetics does not address a patients' physiological or pathological conditions.

Paragraph at page 11, lines 14-19:

Biological pathways, particularly pathways involved in drug actions, *i.e.*, pathways that originate at a drug target (*e.g.*, proteins) and/or are hierarchical, can be identified for use in this invention by several means. Such means for identifying such pathways have been described, in detail, by Stoughton and Friend, U.S. application Serial No. 09/074,983, filed on May 8, 1998, now U.S. Patent No. 5,965,352, and Stoughton and Friend, U.S. application Serial No. 09/179,569, filed on October 27, 1998, which are incorporated herein by reference in their entireties.

Paragraph at page 12, lines 30-35:

The method of invention is based upon the ability to analyze the response of a biological system to the response of pathways. One [particular] particularly useful method for decomposing the drug response is by comparing measurements of changes in the biological state of a cell in response to graded drug exposure with measurements of changes in the biological state of biological pathways that are likely to be involved in the effects of the drug, the changes being in response to graded perturbations of these pathways.

Paragraph at page 24, lines 11-24:

In general, horizontal scaling is expected to be necessary. As discussed above [in Section 5.2], such scaling is necessary because values of the perturbation control parameters for the various candidate biological pathways are likely not to cause saturation responses at the same numerical perturbation control values nor at the same numerical value as the saturation response of the drug exposure. For example, the pathway perturbations may act according to such entirely different mechanisms as the titration of a viral transfection vector expressing a protein from which a pathway originates, or the control parameter of a controllable promoter controlling expression of an originating protein, or the exposure level of a drug of specific known action on an originating protein. The saturating control values of these mechanisms, and indeed their kinetic characteristics, are likely to be all unrelated. All of these mechanisms may be different from the action of the drug of interest. For example, where perturbation action on a cellular constituent from which a pathway originates can be modeled as a Hill function, there is no reason that the various " u_0 " parameters will be the same.

Paragraph at page 25, lines 3-19:

More general horizontal scaling transformations are [possible] characterized by additional parameters. Flexible scaling transformations are possible with a number of parameters small enough, even though nonlinear, to be usefully employed in the minimization procedure of step 504. Multiple scaling parameters for the i 'th pathway are represented herein by " α_i ". Another example of a scaling transformation is a polynomial expansion generalizing the linear transformation of Eqn 3. A simple example of a more general scaling transformation is the previously described Hill function employed according to the following equation.

$$P_{i,l} = \frac{\alpha_i (t_l / \mu_i)^{n_i}}{1 + (t_l / \mu_i)^{n_i}} \quad (4)$$

Again, Eqn. 3 provides the perturbation control value in the i 'th pathway corresponding to the l 'th drug exposure level and is parameterized for each pathway by the three parameters α_i , μ_i , and n_i . The Hill function scaling is more general at least in that it reduces to a linear scaling when n_i is 1 and t_l is much less than μ_i .

Paragraph at page 29, lines 27-34:

According to the preferred method, a residual distribution is constructed by repetitively solving Eqn. 5 with randomized input data and accumulating the residuals to form the empirical residual distribution. Thereby, the constructed empirical residual distribution arises from random data that has the same population statistics as the actual data. In detail, first, either the drug response [date] data or the pathway response data (but not both) are randomized in step 505 with respect to the drug exposure levels or the perturbation control parameters, respectively. This randomization transformation is represented by the following transformation.

Paragraph at page 30, lines 5-13:

In Eqn. 10, Π represents a [perturbation] permutation independently chosen for each cellular constituent. Either the drug response or the each pathway response (but not both) is randomized according to Eqn. 10. Accordingly, the randomized drug or pathway response data are derived from the measured data by independent [perturbations] permutations of the measurement points. Second, Eqn. 5 is then solved by the chosen numerical approximation technique in step 504 and the value of the resulting residual saved. These steps are repeated for enough randomizations to construct a sufficiently significant expected probability distribution of residuals. In order to obtain confidence levels of 99% or better (*i.e.*, a P-value less than 0.01), then more than 100 randomizations are needed.

Paragraph at page 35, lines 19-25:

Where $D_k(t_l)$ is the drug activity on cellular constituent k when the drug is applied at a level t_l ; $R_{i,k}(\alpha_i, t_l)$ is the response of cellular constituent k in pathway i under perturbation (α_i, t_l) (for the scaling transformation of perturbation levels using parameter α_i , see section 5.1, *supra*, or U.S. Patent Application Ser. No. 09/074,983, filed on May 8, 1998, now U.S. Patent No. 5,965,352, previously incorporated by reference). $R_{i,k}(\alpha_i, t_l)$ represents the drug activity on the cellular constituent in pathway i . Drug activity on a cellular constituent k in pathway i is represented as:

$$D_{i,k}(t_l) = R_{i,k}(\alpha_i, t_l) \quad (17)$$

Paragraph at page 35, line 33 through page 36, line 16:

For some embodiments of the invention, the drug activity on a particular pathway is more conveniently represented by a single parameter, rather than a group of responses of cellular constituents. In some preferred embodiments, the drug activity on pathway i , when the drug is applied at the level $[t]$ t_i , is represented by:

$$D_i(t_i) = \sum_k \beta_k R_{i,k}(\alpha_i, t_i) \quad (18)$$

Where β_k is a constant for cellular constituent k . One of skill in the art would appreciate that the selection of constant β_k is dependent upon the unit used in measuring cellular constituent responses. For example, if [both] a cellular constituent response measurement is the activity of an enzyme, while another cellular constituent response measurement is a gene expression ratio, two different β constants can be assigned to the two different cellular constituent types to adjust the difference in units and ranges of the measurements. Selection of the constants in a linear transformation to take account for different units of measurements and different range of variables is well within the skill of those in the art. In one particularly preferred embodiment, where the response of all cellular constituents are measured as the expression ratios (expression under perturbation over expression without perturbation), the β_k is given the value of 1.

Paragraph at page 37, lines 22-34:

One aspect of the invention provides methods for determining the specificity index (SI) of a drug in an *in vitro* system, based upon the drug's activity on target versus off-target pathways. The target and off-target pathways are previously discussed, for example, in Section 5.1, *supra*. The specificity index measurements is particularly useful to evaluate the relative efficacy and toxicity of a drug candidate during the early phase of drug screening. Specificity index is defined herein as the relative activity of a drug against its primary target pathway versus its activity against "off-target" pathways. Methods for determining the activities of a drug on different pathways have been described in detail in the Sections 5.1 and 5.2, *supra*. Some of the methods are also described in Stoughton and Friend, Methods for Identifying Pathways of Drug Action, U.S. Patent Application Ser. No. 09/074,983, filed on May 8, 1998, now U.S. Patent No. 5,965,352, incorporated previously by reference for all

purposes. One of skill in the art would appreciate that the some methods of the invention are limited by particular methods for detecting “on-target” or “off-target” activities of a drug.

Paragraph at page 39, lines 10-13:

Even though it may be difficult to extrapolate a therapeutic index obtained from a model organism to the human or other target systems, the therapeutic index of a particular drug candidate relative to alternative drugs should be indicative of the ranking of those drugs in the target systems, especially when off-target effects of [the] those drugs are similar.

Paragraph at page 39, lines 14-23:

In one preferred method, the threshold is set according to the relationship between toxicity and the pathways involved. For example, if a particular concentration of a drug that induces a particular off-target pathway by two-fold in a model system (such as a yeast model system) and later the drug is found to have toxicity when administered to a patient population at a dose that is equivalent to the concentration, the toxicity threshold may be set as two fold induction for this particular pathway. Similarly, if a particular concentration of a drug that represses a particular target pathway by three folds in a model system and later the drug is found to have a desired therapeutic effect in a patient administered with a dose that is equivalent to the concentration, the therapeutic threshold can be set as three-fold of repression for the particular target pathway.

Paragraph at page 39, line 28 through page 40, line 2:

Example 1 (Section 6, *infra*) illustrates [the] one such embodiment. In this example, the expression of a number of genes are monitored as a wild type yeast culture is subjected to a graded levels of the drug FK506 (Fig. 8A). Similar experiments are repeated with a yeast culture whose CNA1 and CNA2 genes are deleted (Fig. 8B). CNA1 and CNA2 are two components of the calcineurin multi-protein complex. Because the drug FK506 acts upon the calcineurin protein to exert its activity on the calcineurin pathway. Deletion of CNA1 and CNA2 eliminates the primary target pathway for FK506. For a discussion of the yeast model system, see, Cardens et al., 1994, “Yeast as Model T Cells, Prosp. In DRUG [DISCOVER] DISCOVER. DESIGN, 2:103-126.

Paragraph at page 40, lines 19-24:

As discussed in the background section, clinical toxicity signs are difficult to detect. Drug effect or toxicity may not show up as clinical signs before it is too late to make a informed therapeutic decision. The drug response of at least some pathways, however, are relatively faster. Accordingly, this invention provides methods for evaluating the drug effect or toxicity in a patient that undergoes drug therapy using pathway activities rather [that] than clinical signs or individual cellular constituent changes.

Paragraph at page 40, lines 24-33:

In some embodiments, the expression of a large number of [gene] genes in the patient (a human or an animal) is determined while the patient undergoes therapy. The drug responses of the primary target pathway and off-target pathways are determined according to the methods of the invention and other suitable methods. If a patient's primary target pathway does not respond to the drug therapy and/or the off-target pathways respond strongly to the drug therapy, the therapy may be discontinued in favor of alternative treatments. Because the drug response of pathways can sometimes be determined earlier than clinical signs, the method of the invention offers the advantage that clinical decision can be made before clinical toxicity and therapy failure is detected by clinical signs.

Paragraph at page 41, lines 5-12:

In some embodiments, the expression of a large number of genes in a patient is monitored as the patient receives a plurality of perturbations. The perturbation can be a particular drug given at different doses. The drug responses of the target and off target pathways are determined according to the method of invention and other suitable methods. Suitable dosage can be determined so that the drug elicits a strong drug response in the target pathways and a relatively [week] weak response in the off-target pathways. If a strong response in off-target pathways is illicit, the drug is determined to be unsuitable for the particular patient.

Paragraph at page 45, lines 5-15:

Figs. 8A-C illustrate the drug response data generated by a series of FK506 exposures. The horizontal axis is concentrations of the FK506 in logarithmic scale and the vertical axis

is the values of the logarithm of the expression ratio of the genes most affected by FK506 on the vertical axis. Fig. 8A shows the transcriptional response of the yeast genome to a titration of the drug FK506. Fig. [8C] 8B shows the transcriptional response in a different experiment when the drug is applied to a yeast strain in which both components of the calcineurin protein have been removed by deletion of the genes CNA1 and CNA2. Plotted genes have P-Value < 0.03 and $\text{abs}(\text{Log}_{10}(\text{expression ratio})) > 0.3$ at two or more concentrations in the series. P-Value is the probability that the up or down regulation is due to measurement error, as determined from observed statistics of the errors in $\text{Log}_{10}(\text{expression ratio})$.

EXHIBIT B: CLEAN VERSION OF PARAGRAPHS IN THE SPECIFICATION
U.S. APPLICATION SERIAL NO. To be assigned
(ATTORNEY DOCKET NO. 9301-136)

(as amended April 2, 2001)

Paragraph at page 3, lines 7-14:

In a clinical setting, a physician must select, among several drugs, the most effective and safe drug for the patient. In making this decision, the physician needs to know how an particular patient may respond to a drug. One approach to individualized therapy decision making is through pharmacogenetics which relates individual variation in drug response to genetic variations. Pharmacogenetics promises a better understanding of the relationship between genetic variation and drug responses. However, so far, it has only provided limited information related to about 50 - 100 known drug metabolizing genes. In addition, pharmacogenetics does not address a patients' physiological or pathological conditions.

Paragraph at page 11, lines 14-19:

Biological pathways, particularly pathways involved in drug actions, *i.e.*, pathways that originate at a drug target (*e.g.*, proteins) and/or are hierarchical, can be identified for use in this invention by several means. Such means for identifying such pathways have been described, in detail, by Stoughton and Friend, U.S. application Serial No. 09/074,983, filed on May 8, 1998, now U.S. Patent No. 5,965,352, and Stoughton and Friend, U.S. application Serial No. 09/179,569, filed on October 27, 1998, which are incorporated herein by reference in their entireties.

Paragraph at page 12, lines 30-35:

The method of invention is based upon the ability to analyze the response of a biological system to the response of pathways. One particularly useful method for decomposing the drug response is by comparing measurements of changes in the biological state of a cell in response to graded drug exposure with measurements of changes in the biological state of biological pathways that are likely to be involved in the effects of the drug, the changes being in response to graded perturbations of these pathways.

Paragraph at page 24, lines 11-24:

In general, horizontal scaling is expected to be necessary. As discussed above, such scaling is necessary because values of the perturbation control parameters for the various candidate biological pathways are likely not to cause saturation responses at the same numerical perturbation control values nor at the same numerical value as the saturation response of the drug exposure. For example, the pathway perturbations may act according to such entirely different mechanisms as the titration of a viral transfection vector expressing a protein from which a pathway originates, or the control parameter of a controllable promoter controlling expression of an originating protein, or the exposure level of a drug of specific known action on an originating protein. The saturating control values of these mechanisms, and indeed their kinetic characteristics, are likely to be all unrelated. All of these mechanisms may be different from the action of the drug of interest. For example, where perturbation action on a cellular constituent from which a pathway originates can be modeled as a Hill function, there is no reason that the various " u_0 " parameters will be the same.

Paragraph at page 25, lines 3-19:

More general horizontal scaling transformations are characterized by additional parameters. Flexible scaling transformations are possible with a number of parameters small enough, even though nonlinear, to be usefully employed in the minimization procedure of step 504. Multiple scaling parameters for the i 'th pathway are represented herein by " α_i ". Another example of a scaling transformation is a polynomial expansion generalizing the linear transformation of Eqn 3. A simple example of a more general scaling transformation is the previously described Hill function employed according to the following equation.

$$P_{i,l} = \frac{\alpha_i (t_l / \mu_i)^{n_i}}{1 + (t_l / \mu_i)^{n_i}} \quad (4)$$

Again, Eqn. 3 provides the perturbation control value in the i 'th pathway corresponding to the l 'th drug exposure level and is parameterized for each pathway by the three parameters α_i , μ_i , and n_i . The Hill function scaling is more general at least in that it reduces to a linear scaling when n_i is 1 and t_l is much less than μ_i .

Paragraph at page 29, lines 27-34:

According to the preferred method, a residual distribution is constructed by repetitively solving Eqn. 5 with randomized input data and accumulating the residuals to form the empirical residual distribution. Thereby, the constructed empirical residual distribution arises from random data that has the same population statistics as the actual data. In detail, first, either the drug response data or the pathway response data (but not both) are randomized in step 505 with respect to the drug exposure levels or the perturbation control parameters, respectively. This randomization transformation is represented by the following transformation.

Paragraph at page 30, lines 5-13:

In Eqn. 10, Π represents a permutation independently chosen for each cellular constituent. Either the drug response or the each pathway response (but not both) is randomized according to Eqn. 10. Accordingly, the randomized drug or pathway response data are derived from the measured data by independent permutations of the measurement points. Second, Eqn. 5 is then solved by the chosen numerical approximation technique in step 504 and the value of the resulting residual saved. These steps are repeated for enough randomizations to construct a sufficiently significant expected probability distribution of residuals. In order to obtain confidence levels of 99% or better (*i.e.*, a P-value less than 0.01), then more than 100 randomizations are needed.

Paragraph at page 35, lines 19-25:

Where $D_k(t_l)$ is the drug activity on cellular constituent k when the drug is applied at a level t_l ; $R_{i,k}(\alpha_i, t_l)$ is the response of cellular constituent k in pathway i under perturbation (α_i, t_l) (for the scaling transformation of perturbation levels using parameter α_i , see section 5.1, *supra*, or U.S. Patent Application Ser. No. 09/074,983, filed on May 8, 1998, now U.S. Patent No. 5,965,352, previously incorporated by reference). $R_{i,k}(\alpha_i, t_l)$ represents the drug activity on the cellular constituent in pathway i . Drug activity on a cellular constituent k in pathway i is represented as:

$$D_{i,k}(t_l) = R_{i,k}(\alpha_i, t_l) \quad (17)$$

Paragraph at page 35, line 33 through page 36, line 16:

For some embodiments of the invention, the drug activity on a particular pathway is more conveniently represented by a single parameter, rather than a group of responses of cellular constituents. In some preferred embodiments, the drug activity on pathway i , when the drug is applied at the level t_i , is represented by:

$$D_i(t_i) = \sum_k \beta_k R_{i,k}(\alpha_i, t_i) \quad (18)$$

Where β_k is a constant for cellular constituent k . One of skill in the art would appreciate that the selection of constant β_k is dependent upon the unit used in measuring cellular constituent responses. For example, if a cellular constituent response measurement is the activity of an enzyme, while another cellular constituent response measurement is a gene expression ratio, two different β constants can be assigned to the two different cellular constituent types to adjust the difference in units and ranges of the measurements. Selection of the constants in a linear transformation to take account for different units of measurements and different range of variables is well within the skill of those in the art. In one particularly preferred embodiment, where the response of all cellular constituents are measured as the expression ratios (expression under perturbation over expression without perturbation), the β_k is given the value of 1.

Paragraph at page 37, lines 22-34:

One aspect of the invention provides methods for determining the specificity index (SI) of a drug in an *in vitro* system, based upon the drug's activity on target versus off-target pathways. The target and off-target pathways are previously discussed, for example, in Section 5.1, *supra*. The specificity index measurements is particularly useful to evaluate the relative efficacy and toxicity of a drug candidate during the early phase of drug screening. Specificity index is defined herein as the relative activity of a drug against its primary target pathway versus its activity against "off-target" pathways. Methods for determining the activities of a drug on different pathways have been described in detail in the Sections 5.1 and 5.2, *supra*. Some of the methods are also described in Stoughton and Friend, Methods for Identifying Pathways of Drug Action, U.S. Patent Application Ser. No. 09/074,983, filed on May 8, 1998, now U.S. Patent No. 5,965,352, incorporated previously by reference for all purposes. One of skill in the art would appreciate that the some methods of the invention are limited by particular methods for detecting "on-target" or "off-target" activities of a drug.

Paragraph at page 39, lines 10-13:

Even though it may be difficult to extrapolate a therapeutic index obtained from a model organism to the human or other target systems, the therapeutic index of a particular drug candidate relative to alternative drugs should be indicative of the ranking of those drugs in the target systems, especially when off-target effects of those drugs are similar.

Paragraph at page 39, lines 14-23:

In one preferred method, the threshold is set according to the relationship between toxicity and the pathways involved. For example, if a particular concentration of a drug that induces a particular off-target pathway by two-fold in a model system (such as a yeast model system) and later the drug is found to have toxicity when administered to a patient population at a dose that is equivalent to the concentration, the toxicity threshold may be set as two fold induction for this particular pathway. Similarly, if a particular concentration of a drug that represses a particular target pathway by three folds in a model system and later the drug is found to have a desired therapeutic effect in a patient administered with a dose that is equivalent to the concentration, the therapeutic threshold can be set as three-fold of repression for the particular target pathway.

Paragraph at page 39, line 28 through page 40, line 2:

Example 1 (Section 6, *infra*) illustrates one such embodiment. In this example, the expression of a number of genes are monitored as a wild type yeast culture is subjected to a graded levels of the drug FK506 (Fig. 8A). Similar experiments are repeated with a yeast culture whose CNA1 and CNA2 genes are deleted (Fig. 8B). CNA1 and CNA2 are two components of the calcineurin multi-protein complex. Because the drug FK506 acts upon the calcineurin protein to exert its activity on the calcineurin pathway. Deletion of CNA1 and CNA2 eliminates the primary target pathway for FK506. For a discussion of the yeast model system, see, Cardens et al., 1994, "Yeast as Model T Cells, Prosp. In DRUG DISCOVERY. DESIGN, 2:103-126.

Paragraph at page 40, lines 19-24:

As discussed in the background section, clinical toxicity signs are difficult to detect. Drug effect or toxicity may not show up as clinical signs before it is too late to make a

informed therapeutic decision. The drug response of at least some pathways, however, are relatively faster. Accordingly, this invention provides methods for evaluating the drug effect or toxicity in a patient that undergoes drug therapy using pathway activities rather than clinical signs or individual cellular constituent changes.

Paragraph at page 40, lines 24-33:

In some embodiments, the expression of a large number of genes in the patient (a human or an animal) is determined while the patient undergoes therapy. The drug responses of the primary target pathway and off-target pathways are determined according to the methods of the invention and other suitable methods. If a patient's primary target pathway does not respond to the drug therapy and/or the off-target pathways respond strongly to the drug therapy, the therapy may be discontinued in favor of alternative treatments. Because the drug response of pathways can sometimes be determined earlier than clinical signs, the method of the invention offers the advantage that clinical decision can be made before clinical toxicity and therapy failure is detected by clinical signs.

Paragraph at page 41, lines 5-12:

In some embodiments, the expression of a large number of genes in a patient is monitored as the patient receives a plurality of perturbations. The perturbation can be a particular drug given at different doses. The drug responses of the target and off target pathways are determined according to the method of invention and other suitable methods. Suitable dosage can be determined so that the drug elicits a strong drug response in the target pathways and a relatively weak response in the off-target pathways. If a strong response in off-target pathways is illicit, the drug is determined to be unsuitable for the particular patient.

Paragraph at page 45, lines 5-15:

Figs. 8A-C illustrate the drug response data generated by a series of FK506 exposures. The horizontal axis is concentrations of the FK506 in logarithmic scale and the vertical axis is the values of the logarithm of the expression ratio of the genes most affected by FK506 on the vertical axis. Fig. 8A shows the transcriptional response of the yeast genome to a titration of the drug FK506. Fig. 8B shows the transcriptional response in a different experiment when the drug is applied to a yeast strain in which both components of the calcineurin protein

have been removed by deletion of the genes CNA1 and CNA2. Plotted genes have P-Value < 0.03 and $\text{abs}(\text{Log10}(\text{expression ratio})) > 0.3$ at two or more concentrations in the series. P-Value is the probability that the up or down regulation is due to measurement error, as determined from observed statistics of the errors in $\text{Log10}(\text{expression ratio})$.

EXHIBIT C: MARKED VERSION OF CLAIMS
U.S. APPLICATION SERIAL NO. To be assigned
(ATTORNEY DOCKET NO. 9301-136)

(as amended April 2, 2001)

1. (Amended) A method for evaluating specificity of a drug comprising comparing activity of said drug against its target pathway (D_{target}) in a biological sample and activity of said drug against at least one off-target pathway ($D_{off-target}$) in said biological sample, wherein said D_{target} and $D_{off-target}$ are based on measurements of a plurality of cellular constituents.

12. (Amended) A method for evaluating specificity of a drug comprising:

- a) measuring activity of said drug against its target pathway to obtain a target activity (D_{target});
- b) measuring activity of said drug against at least one pathway other than said target pathway to obtain at least one off-target activity ($D_{off-target}$); and
- c) determining said specificity by comparing said target activity and said off-target activity;

wherein said D_{target} and $D_{off-target}$ are based on measurements of a plurality of cellular constituents.

23. (Amended) A method of determining therapeutic index of a drug in a biological sample comprising:

determining said therapeutic index according to the formula: $[SI] TI = C_{target} / C_{off-target}$, wherein C_{target} is a minimum effective concentration needed to induce a threshold response in a target pathway and $C_{off-target}$ is the minimum toxic concentration needed to induce a threshold response in at least one off-target pathway.

24. (Amended) The method of claim 23 wherein said C_{target} and $C_{off-target}$ are measured according to a method comprising:

- a) applying a plurality of levels of said drug to said biological sample and measuring a plurality of cellular constituents at each level of said drug in said biological sample to obtain a first profile of graded drug response;

b) applying said plurality of levels of said drug to a test sample, wherein said test sample is the same as said biological sample except that said target pathway is not functional, and measuring a plurality of cellular [constituents] constituents in said test sample at each level of said drug, to obtain a second profile of graded drug response; and

c) determining said C_{target} and $C_{off-target}$ by comparing said first and second profiles.

34. (Amended) A method of determining a therapeutic index of a drug in a biological sample comprising:

a) applying a plurality of levels of said drug to said biological sample;

b) determining a minimum effective concentration (C_{target}) needed to induce a threshold response in a target pathway, wherein said drug exerts its pharmacological activity through said target pathway;

c) determining a minimum toxic concentration ($C_{off-target}$) needed to induce a threshold response in at least one off-target pathway; and

d) determining said therapeutic index according to the formula: $[SI] TI = C_{target} / C_{off-target}$

36. (Amended) The method of claim 35 [herein] wherein said plurality of cellular constituents are transcripts of a plurality of genes.

New claims 64-70 have been added.

EXHIBIT D: CLEAN VERSION OF PENDING CLAIMS

U.S. APPLICATION SERIAL NO. To be assigned

(ATTORNEY DOCKET NO. 9301-136)

(as amended April 2, 2001)

1. (Amended) A method for evaluating specificity of a drug comprising comparing activity of said drug against its target pathway (D_{target}) in a biological sample and activity of said drug against at least one off-target pathway ($D_{off-target}$) in said biological sample, wherein said D_{target} and $D_{off-target}$ are based on measurements of a plurality of cellular constituents.

2. The method of Claim 1 wherein said D_{target} and $D_{off-target}$ are measured according to a method comprising:

a) applying a plurality of levels of said drug to said biological sample and measuring a plurality of cellular constituents in said biological sample at each level of said drug to obtain a first profile of graded drug response;

b) applying said plurality of levels of said drug to a test sample, wherein said test sample is the same as said biological sample except that said target pathway is not functional, and measuring said plurality of cellular constituents in said test sample at each level of said drug to obtain a second profile of graded drug response; and

c) determining said D_{target} and $D_{off-target}$ by comparing said first and second profiles.

3. The method of claim 2 wherein said biological sample is a yeast cell and said test sample is a yeast cell with a critical gene in said target pathway being deleted.

4. The method of claim 2 wherein said biological sample is a mammalian cell and said test sample is a mammalian cell with a critical gene in said target pathway being deleted.

5. The method of claim 2 wherein said biological sample is an animal and said test sample is a transgenic animal with a critical gene in said target pathway being made nonfunctional.

6. The method of claim 2 wherein said plurality of cellular constituents are transcripts of a plurality of genes.

7. The method of claim 2 wherein said plurality of cellular constituents are proteins.

8. The method of claim 1 wherein said D_{target} and $D_{off-target}$ are measured according to a method comprising:

- a) perturbing said target pathway and/or said off target pathway in said biological sample to obtain a perturbation profile consisting of a plurality of cellular constituent measurements;
- b) applying a plurality of levels of said drug to said biological sample to obtain a drug response profile consisting of a plurality of cellular constituent measurements at each level of said drug; and
- c) decomposing said D_{target} and $D_{off-target}$ by comparing said drug response profile and said perturbation profile.

9. The method of claim 8 wherein said plurality of cellular constituents are transcripts of a plurality of genes.

10. The method of claim 8 wherein said plurality of cellular constituents are proteins.

11. The method of claim 1 wherein said determining step comprises calculating a specificity index (SI) according to the following formulae:

$$SI = \frac{n \cdot D_{target}}{\sum D_{off-target}}$$

wherein: n is the number of said off-target pathways.

12. (Amended) A method for evaluating specificity of a drug comprising:

- a) measuring activity of said drug against its target pathway to obtain a target activity (D_{target});
- b) measuring activity of said drug against at least one pathway other than said target pathway to obtain at least one off-target activity ($D_{off-target}$); and
- c) determining said specificity by comparing said target activity and said off-target activity;

wherein said D_{target} and $D_{off-target}$ are based on measurements of a plurality of cellular constituents.

13. The method of claim 12 wherein said D_{target} and $D_{off-target}$ are measured according to a method comprising:

- a) applying a plurality of levels of said drug to said biological sample and measuring a plurality of cellular constituents in said biological sample at each level of said drug to obtain a first profile of graded drug response;
- b) applying said plurality of levels of said drug to a test sample, wherein said test sample is the same as said biological sample except that said target pathway is not functional, and measuring said plurality of cellular constituents in said test sample at each level of said drug to obtain a second profile of graded drug response; and
- c) determining said D_{target} and $D_{off-target}$ by comparing said first and second profiles.

14. The method of claim 13 wherein said plurality of cellular constituents are transcripts of a plurality of genes.

15. The method of claim 13 wherein said plurality of cellular constituents are proteins.

16. The method of claim 13 wherein said biological sample is a yeast cell and said test sample is a yeast cell with a critical gene in said target pathway being deleted.

17. The method of claim 13 wherein said biological sample is a mammalian cell and said test sample is a mammalian cell with a critical gene in said target pathway being deleted.

18. The method of claim 13 wherein said biological sample is an animal and said test sample is a transgenic animal with a critical gene in said target pathway being made nonfunctional.

19. The method of claim 12 wherein said D_{target} and $D_{off-target}$ are measured according to a method comprising:

- a) perturbing said target pathway and/or said off target pathway in said biological sample to obtain a perturbation profile consisting of a plurality of cellular constituent measurements;
- b) applying a plurality of levels of said drug to said biological sample to obtain a drug response profile consisting of a plurality of cellular constituent measurements at each level of said drug; and
- c) decomposing said D_{target} and $D_{off-target}$ by comparing said drug response profile and said perturbation profile.

20. The method of claim 19 wherein said plurality of cellular constituents are transcripts of a plurality of genes.

21. The method of claim 20 wherein said plurality of cellular constituents are proteins.

22. The method of claim 12 wherein said determining step comprises calculating a specificity index (SI) according to the following formulae:

$$SI = \frac{n \bullet D_{target}}{\sum D_{off-target}}$$

wherein: n is the number of said off-target pathways.

23. (Amended) A method of determining therapeutic index of a drug in a biological sample comprising:

determining said therapeutic index according to the formula: $TI = C_{target} / C_{off-target}$, wherein C_{target} is a minimum effective concentration needed to induce a threshold response in a target pathway and $C_{off-target}$ is the minimum toxic concentration needed to induce a threshold response in at least one off-target pathway.

24. (Amended) The method of claim 23 wherein said C_{target} and $C_{off-target}$ are measured according to a method comprising:

a) applying a plurality of levels of said drug to said biological sample and measuring a plurality of cellular constituents at each level of said drug in said biological sample to obtain a first profile of graded drug response;

b) applying said plurality of levels of said drug to a test sample, wherein said test sample is the same as said biological sample except that said target pathway is not functional, and measuring a plurality of cellular constituents in said test sample at each level of said drug, to obtain a second profile of graded drug response; and

c) determining said C_{target} and $C_{off-target}$ by comparing said first and second profiles.

25. The method of claim 24 wherein said plurality of cellular constituents are transcripts of a plurality of genes.

26. The method of claim 24 wherein said plurality of cellular constituents are proteins.

27. The method of claim 24 wherein said biological sample is a yeast cell and said test sample is a yeast cell with a critical gene in said target pathway being deleted.

28. The method of claim 24 wherein said biological sample is a mammalian cell and said test sample is a mammalian cell with a critical gene in said target pathway being deleted.

29. The method of claim 24 wherein said biological sample is an animal and said test sample is a transgenic animal with a critical gene in said target pathway being made nonfunctional.

30. The method of claim 24 wherein said target threshold response is at least two fold induction or repression of a plurality of cellular constituents in said target pathway.

31. The method of claim 24 wherein said off-target threshold response is at least two fold induction or repression of a plurality of cellular constituents in said off-target pathway.

32. The method of claim 23 wherein said target threshold response is a response that is sufficient to have a therapeutic effect.

33. The method of claim 23 wherein said off-target threshold response is a response that is sufficient to constitute a toxic effect.

34. (Amended) A method of determining a therapeutic index of a drug in a biological sample comprising:

- a) applying a plurality of levels of said drug to said biological sample;
- b) determining a minimum effective concentration (C_{target}) needed to induce a threshold response in a target pathway, wherein said drug exerts its pharmacological activity through said target pathway;
- c) determining a minimum toxic concentration ($C_{\text{off-target}}$) needed to induce a threshold response in at least one off-target pathway; and
- d) determining said therapeutic index according to the formula: $TI = C_{\text{target}} / C_{\text{off-target}}$.

35. The method of claim 34 wherein said C_{target} and $C_{\text{off-target}}$ are measured according to a method comprising:

- a) applying a plurality of levels of said drug to said biological sample and measuring a plurality of cellular constituents at each level of said drug in said biological sample to obtain a first profile of graded drug response;
- b) applying said plurality of levels of said drug to a test sample, wherein said test sample is the same as said biological sample except that said target pathway is not functional, and measuring a plurality of cellular constituents in said test sample at each level of said drug to obtain a second profile of graded drug response; and
- c) determining said C_{target} and $C_{\text{off-target}}$ by comparing said first and second profiles.

36. (Amended) The method of claim 35 wherein said plurality of cellular constituents are transcripts of a plurality of genes.

37. The method of claim 35 wherein said plurality of cellular constituents are proteins.

38. The method of claim 35 wherein said biological sample is a yeast cell and said test sample is a yeast cell with a critical gene in said target pathway being deleted.

39. The method of claim 36 wherein said biological sample is a mammalian cell and said test sample is a mammalian cell with a critical gene in said target pathway being deleted.

40. The method of claim 36 wherein said biological sample is an animal and said test sample is a transgenic animal with a critical gene in said target pathway being made nonfunctional.

41. The method of claim 35 wherein said target threshold response is at least two fold induction or repression of a plurality of cellular constituents in said target pathway.

42. The method of claim 35 wherein said off-target threshold response is at least two fold induction or repression of a plurality of cellular constituents in said off-target pathway.

43. The method of claim 35 wherein said target threshold response is a response that is sufficient to have a therapeutic effect.

44. The method of claim 35 wherein said off-target threshold response is a response that is sufficient to constitute a toxic effect.

64. (New) A method for evaluating specificity of a drug, said method comprising:

(a) decomposing a drug response profile into one or a combination of pathway response profiles, wherein said drug response profile comprises measurements of a plurality of cellular constituents in a biological sample in response to said drug over a plurality of levels of drug exposure, and each said pathway response profile comprises measurements of a plurality of cellular constituents at a plurality of levels of perturbation to a biological pathway; and

(b) comparing, among said one or a combination of pathway response profiles, the pathway response profiles for the one or more biological pathways associated with therapeutic effects of the drug with the pathway response profiles for the one or more

biological pathways that are associated with one or more non-therapeutic effects of the drug, thereby comparing activity of said drug on its target pathway (D_{target}) and at least one off-target pathway ($D_{off-target}$) and evaluating specificity of said drug.

65. (New) The method of claim 64, further comprising transforming said levels of drug exposure into said levels of perturbation by a horizontal scaling transformation.

66. (New) The method of claim 65, wherein said horizontal scaling transformation is a linear transformation.

67. (New) The method of claim 65, wherein said decomposing comprises determining said scaling transformation such that said drug response profile is represented by said one or a combination of pathway response profiles.

68. (New) The method of claim 67, wherein said determining is by a method comprising least squares minimizing the residue between said drug response profile and said one or a combination of pathway response profiles.

69. (New) The method of claim 64, wherein values of said measurements of a plurality of cellular constituents have been converted into cellular constituent set values.

70. (New) A method for evaluating specificity of a drug, said method comprising decomposing a drug response profile into one or a combination of pathway response profiles, wherein said drug response profile comprises measurements of a plurality of cellular constituents in a biological sample in response to said drug over a plurality of levels of drug dosage, and each said pathway response profile comprises measurements of a plurality of cellular constituents at a plurality of levels of perturbation to a biological pathway, thereby evaluating specificity of said drug.